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Effects of Sidestream Smoke Exposure and Age on Pulmonary Function and Airway Reactivity in Developing Rats

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Summary. Children exposed to environmental tobacco smoke (ETS) in their homes have increased cough, respiratory illness, airway obstruction, and hyperreactivity. Since an animal model is needed to understand the mechanism by which this occurs, our study was designed to determine if immature rats develop airway obstruction and increased airway reactivity when exposed to sidestream smoke (SSS, respirable suspended particulate concentration 1.00 ± 0.03 mg m^3, CO concentration 6.48 ± 0.29 ppm). In the first of 3 studies, rats were exposed to filtered air (FA) or SSS for 6 hr day, 5 days/week from day 2 to week 8 or week 15 of life (n = 6–8 in each group). SSS exposure did not change lung resistance (R_L), dynamic lung compliance (C_{Ldyn}), lung weight/body weight ratio (LW/BW), pulmonary artery pressure (P_{PA}), body weight, or airway reactivity to methacholine (all $P > 0.2$, 2-way ANOVA). Regardless of exposure, lungs from younger rats were relatively heavier and more reactive to methacholine than lungs from older rats ($P < 0.05$, 2-way ANOVA). In the second study, 15-week-old rats were exposed to FA or SSS for 3 hr or for 4 days (6 hr/day, n = 6 in each group). SSS exposure again had no effect on C_{Ldyn}, R_L, LW/BW, P_{PA}, or airway reactivity to methacholine (all $P > 0.2$, ANOVA). In the third study, rats were exposed to FA or SSS from day 2 to week 11 of life (n = 7 in each group). SSS exposure reduced airway ($P = 0.004$) but not pulmonary artery ($P = 0.63$) reactivity to serotonin. We conclude that (1) SSS exposure to the immature rats did not mimic the effects of ETS seen in children, (2) younger rats had greater muscarinic airway reactivity than older rats, and (3) serotonin may play a role in ETS-induced lung problems. **Pediatr Pulmonol. 1993; 16:281–288.**

Key words: Dynamic lung compliance, lung resistance, pulmonary artery pressure; methacholine challenge; lung weight/body weight ratio.

INTRODUCTION

A number of studies have demonstrated that children living in homes with environmental tobacco smoke (ETS) exposure have increased respiratory problems. Bonham and Wilson[1] showed in a survey of 37,000 households, that children in homes with two smokers had 0.9 year more bed disability days due to respiratory illness than children in homes with no smokers. Ekwo et al.[2], evaluating 1355 children, found that those exposed to ETS had increased cough with colds and increased risk of hospitalizations for respiratory illness in infancy. In a study of 6000 children, Bland et al.[3] also showed increased cough in children of smokers and Dodge[4] found that children with ETS exposure had increased cough, wheeze, and sputum production.

Evaluation of pulmonary function of children raised under ETS exposure has shown that they have decreased forced expiratory volume in 1 second (FEV_1),[5,6] FEV_1 to forced vital capacity ratio (FEV_1/FVC),[7] forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}),[8] and maximal midexpiratory flow (MMEF),[5] suggesting that their airways were obstructed. Infants exposed to ETS in the home also exhibited increased airway reactivity.[9,10]

Children exposed to ETS have an increased incidence of asthma,[8,11] an increased likelihood of using asthma medications, and an earlier (first year of life) onset of asthma.[11] In children with asthma, ETS exposure is asso-

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ciated with more severe asthma and greater airway reactivity to histamine[12] and cold air.[6]

Together, these studies demonstrate that children exposed to ETS have increased respiratory symptoms, airways obstruction, increased airway reactivity, and a higher incidence and severity of clinical asthma. However, since many studies suggest that it is the mother's smoking status which primarily effects the child's pulmonary function,[5,8,11,12] the effects of prenatal smoke exposure cannot be easily separated from the effects of ETS generated by the primary caretaker.

This study was designed to determine if exposing the developing rat to sidestream smoke (SSS, a component of ETS) could function as a model for studying the effects of ETS exposure on children in the absence of in utero exposure to smoke. Specifically, this study was designed to determine if SSS exposure to the developing rat (1) causes airway obstruction and airway hyperreactivity to methacholine and/or serotonin, and (2) if these effects are dependent on age and/or chronicity of exposure.

MATERIALS AND METHODS

Protocol #1 for Studying the Effect of Age and Chronic SSS Exposure on Lung Function and Reactivity to Methacholine

Female Sprague–Dawley rats were exposed to sidestream smoke (SSS) or filtered air (FA) from day 2 of life until either 8 or 15 weeks of age (n = 6–8 in each group). Their lungs were then removed and placed in an isolated perfused lung system where measurements were made of dynamic lung compliance ($C_{L,dyn}$), pulmonary resistance (R_L), pulmonary artery pressure (P_{PA}), reactivity to methacholine, and lung weight.

Protocol #2 for Studying the Effect of Acute SSS Exposure on Lung Function and Reactivity to Methacholine

Female Sprague-Dawley rats, 15 weeks of age, were exposed to SSS or FA for 3 hr or for 4 days (n = 6 in each group). Their lungs were then removed and hung in an isolated perfused lung system where measurements were made of $C_{L,dyn}$, R_L, P_{PA}, reactivity to methacholine, and lung weight.

Protocol #3 for Studying the Effect of Chronic SSS Exposure on Reactivity to Serotonin

Female Sprague–Dawley rats were exposed to SSS or FA from day 2 of life until 11 weeks of age (n = 7 in each group). Their lungs were then removed and hung in an isolated perfused lung system where measurements were made of $C_{L,dyn}$, R_L, P_{PA}, reactivity to serotonin, and lung weight.

Chronic Exposure Protocol

Timed pregnant Sprague–Dawley rats (Charles River, Wilmington, MA) were allowed to litter. At 2 days of life, the pups were randomly divided into groups. Half the groups received SSS exposure for 6 hr/day, 5 days per week and FA the rest of the time. The other half of the groups received FA all the time. At 21 days, the pups were weaned and continued on their exposure regimen.

Acute Exposure Protocols

Rats raised in FA were (1) placed in SSS for 3 hr and studied within 2 hr or (2) placed in SSS for 6 hr/day for 4 days and studied the following day, or (3) maintained in a FA environment.

Generation of SSS Exposure Atmosphere

Dilute SSS was generated by a modified ADL II smoke exposure system (Oakridge National Laboratory) using conditioned 1R4F cigarettes from the Tobacco and Health Research Institute of the University of Kentucky. Cigarettes were smoked under FTC conditions 2 at a time at a rate of 1 puff (35 mL, 2 seconds duration) per minute. The mainstream smoke was collected on a filter and discarded. The SSS was diluted with filtered air in a mixing chamber than passed into the exposure chamber. The exposure chamber was characterized by a relative humidity of 29.9 ± 7.4%, temperature of 22.6 ± 1.4°C, respirable suspended particulate (RSP) concentration of 1.00 ± 0.03 mg m^{-3}, and carbon monoxide (CO) concentration of 6.48 ± 0.29 ppm (mean ± SD).

Isolated Perfused Lung System

As we have done previously, lungs were studied in an isolated perfused system to separate them from the effects of circulating blood components and central neural control.[13] Rats were anesthetized with 150 mg/kg pentobarbital IP. The trachea was cannulated and the rat ventilated with room air at a rate of 80 breaths/min and a tidal volume (V_T) of 3 mL. The chest was opened and 500 units of heparin injected into the right ventricle. The inspired gas was then changed to 5% CO_2 mixed with room air, the right ventricle incised, and a canula placed into the main pulmonary artery. The left ventricle was incised and the lungs were washed free of blood with a warmed (37°C) Krebs–Ringer bicarbonate buffer (NaCl 119 mM, $CaCl_2$ 0.5 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 24.9 mM, KH_2PO_4 1.2 mM, albumin 4.5%, glucose 0.1%, pH 7.30–7.40). The left atrium was then cannulated, the lung dissected free, and hung by the trachea in a water vapor-saturated chamber.

Warmed (37°C), humidified gas (95% air and 5% CO_2) was supplied at a bias flow of 900 mL/min. The lung was ventilated with transpulmonary pressure fluctuating between −2.5 and −10 cm H_2O at a rate of 60

TABLE 1—Effect of Age and SSS Exposure on Body Weight, Lung Weight, P_{PA}, and Pulmonary Mechanics (Mean ± SEM)

	8 weeks (n = 6–8)		15 weeks (n = 6–8)		P^*	P^*
	FA	SSS	FA	SSS	(age)	(SSS)
Body wt (g)	212 ± 4	216 ± 9	307 ± 10	305 ± 10	0.0001	0.91
Lung wt/body wt (%)	0.488 ± 0.031	0.492 ± 0.026	0.365 ± 0.023	0.392 ± 0.021	0.0004	0.58
P_{PA} (mm Hg)	15.3 ± 1.5	19.6 ± 2.9	12.4 ± 0.9	12.0 ± 0.6	0.008	0.29
$C_{L,dyn}$ (mL/cm H_2O)	0.300 ± 0.033	0.384 ± 0.051	0.470 ± 0.046	0.484 ± 0.079	0.02	0.36
R_L (cm H_2O/mL/s)	0.209 ± 0.012	0.196 ± 0.018	0.180 ± 0.020	0.182 ± 0.024	0.28	0.25

*P values for 2-way ANOVA (no interactions were seen).

FA, filtered air; SSS, sidestream smoke; P_{PA}, pulmonary artery pressure; $C_{L,dyn}$, dynamic lung compliance; R_L, lung resistance.

breaths/min and was allowed to stabilize for 60 min. The lung was hyperinflated under 20 cm H_2O pressure for 10 seconds at 15 minute intervals during the stabilization period to prevent and reverse atelectasis. A differential pressure transducer (Valindyne, Northridge, CA) measured transpulmonary pressure and a Fleisch 0000 pneumotachograph (OEM, Richmond VA), via a second pressure transducer measured airflow. All voltages were passed through carrier demodulators (Valindyne, Northridge, CA) into a Modular Instruments Data Acquisition System (Malvern, PA) where R_L and $C_{L,dyn}$ (method of Amdur and Mead, 14), and V_T were calculated. The average value over a 5-sec period was used except for dose–response curves where the maximum value was used for R_L, and the minimum value used for $C_{L,dyn}$.

The lungs were perfused with the warmed Krebs–Ringer bicarbonate buffer in a recirculating fashion via a peristaltic pump at a rate of 0.04 mL/g body weight/min. The pH of the perfusate was maintained between 7.2 and 7.4 by the addition of $NaHCO_3$ if needed. The P_{PA} was measured with a pressure transducer (Gould; Cupertino, CA), the voltage passed into the Modular Instruments System. For methacholine and serotonin dose–response curves, increasing doses of drug (at quarter log intervals) were injected in 100 μL bolus volumes every 45 sec into a port of the pulmonary artery catheter situated about 70 cm from the heart. Bolus injection of 100 μL normal saline did not change P_{PA} or pulmonary function.

At the end of the experiments, the lungs were weighed. Lung weight was divided by total body weight to correct for differences in animal size.

Statistical Evaluation

For protocol #1, most of the variables in the four exposure conditions (FA 8WK, SSS 8WK, FA 15WK, SSS 15WK) were compared using a 2-way analysis of variance (ANOVA, Statview, Brainpower) looking at the effects of exposure, age, and their interaction. The values in the methacholine dose response curve were log transformed to equalize variance and analyzed with a 2-way multivariate repeated measures ANOVA (SAS/STAT, SAS Institute) looking at the effect of exposure, age, dose, and their interactions. For protocol #2 the variables in the 3 exposure conditions (FA, SSS 3 hr, SSS 4 day) were compared using a 1-way ANOVA (Statview, Brainpower). For Protocol #3, most of the variables in the two conditions (FA and SSS) were compared using an unpaired t test (SAS/STAT, SAS Institute). The values for the serotonin dose–response curves were log transformed to equalize variance then analyzed with a 1-way multivariate repeated measures ANOVA (SAS/STAT, SAS Institute) looking at the effect of exposure, dose, and their interaction. A type I error < 0.05 was considered significant.

RESULTS

Protocol #1 for Studying the Effect of Age and Chronic SSS Exposure on Lung Function and Reactivity to Methacholine

Exposure of the developing rats to SSS did not change body weight, lung weight/body weight, baseline pulmonary function, P_{PA}, after either 8 or 15 weeks exposure (Table 1). Similarly, airway reactivity to methacholine in SSS-exposed rats did not differ from that in FA-exposed rats of the same age (Fig. 1).

A number of age related-effects were found: 15-week-old rats had 43% greater body weight, 23% lower lung weight/body weight ratio, 41% greater $C_{L,dyn}$, 29% lower PA pressure (Table 1), and less airway reactivity to methacholine than 8-week-old rats (Fig. 1).

Protocol #2 for Studying the Effect of Acute SSS Exposure on Lung Function and Reactivity to Methacholine

Neither 3 hr nor 4 days of SSS exposure affected the $C_{L,dyn}$, R_L, P_{PA}, lung weight/body weight ratio, or airway reactivity to methacholine as defined as the dose required to increase R_L 2-fold (Table 2).

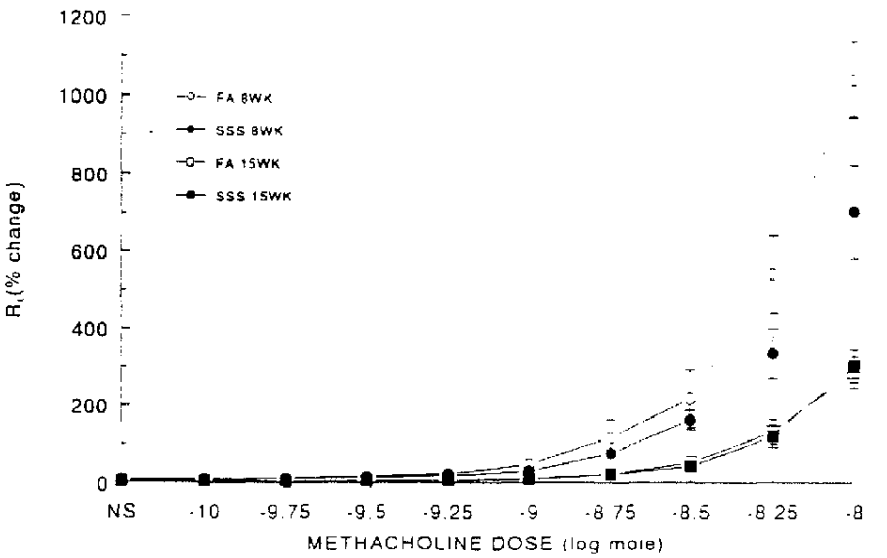

Fig. 1. Methacholine-induced changes of lung resistance (R_L) in rats exposed since day 2 of life to either filtered air (FA) (open symbols) or to sidestream smoke (SSS) (closed symbols) and studied at either 8 (circles) or 15 (squares) weeks of life (n = 6–8 in each group). Methacholine increased R_L (P = 0.0001). Older lungs were less reactive than younger lungs (P = 0.0001). SSS had no effect on lung reactivity to methacholine (P = 0.23). Statistics by 2-way multivariate repeated measures ANOVA on log transformed data.

TABLE 2—Effect of 3 Hour and 4 Day Sidestream Smoke (SSS) Exposure on Lung Weight, Pulmonary Artery Pressure (P_{PA}), Pulmonary Mechanics, and Reactivity (Mean ± SEM)

	FA (n = 6)	SSS 3 hr (n = 6)	SSS 4 day (n = 6)	P*
Lung wt/body wt ($\times 10^{-3}$)	3.65 ± 0.02	3.83 ± 0.02	3.85 ± 0.02	0.84
P_{PA} (mm Hg)	12.4 ± 0.9	11.9 ± 0.5	13.6 ± 1.8	0.61
$C_{L,dyn}$ (mL/cm H_2O)	0.47 ± 0.05	0.46 ± 0.08	0.58 ± 0.08	0.44
R_L (cm H_2O/mL/s)	0.18 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.39
PD_{2x} (−log mol)	8.13 ± 0.06	8.22 ± 0.07	8.16 ± 0.16	0.84

$C_{L,dyn}$, dynamic lung compliance; R_L, lung resistance; PD_{2x}, dose of methacholine required to increase R_L 2-fold.

*P values for 1-way ANOVA.

Protocol #3 for Studying the Effect of Chronic SSS Exposure on Reactivity to Serotonin

Exposure of the developing rat to SSS decreased airway reactivity to serotonin (Fig. 2) but did not change pulmonary artery reactivity to serotonin (Fig. 3). Lung weight/body weight ratio after serotonin in the FA-exposed group did not differ from that in the SSS-exposed group (0.413% ± 0.018% vs 0.507% ± 0.048%; mean ± SEM; P = 0.12, t test).

DISCUSSION

The major findings of this study are (1) chronic exposure of the developing rat to SSS did not alter body growth, lung weight/body weight ratio, R_L, $C_{L,dyn}$, P_{PA}, or airway reactivity to methacholine. However, chronic SSS exposure reduced airway reactivity, but not pulmonary artery reactivity to serotonin; (2) acute (3 hr, 4 day) exposures of the rat to SSS also did not alter body weight, lung weight/body weight ratio, R_L, $C_{L,dyn}$, or airway reactivity to methacholine; (3) regardless of exposure, younger rats showed greater relative lung weight and airway reactivity to methacholine than older rats.

The epidemiologic data suggested that children raised in homes of smokers showed airway obstruction[5–7] and increased airway reactivity.[8,9,10,12] Thus, we expected that the rats chronically exposed to SSS in this study would have decreased lung function and increased airway reactivity to methacholine which they did not. There are several possible explanations for this.

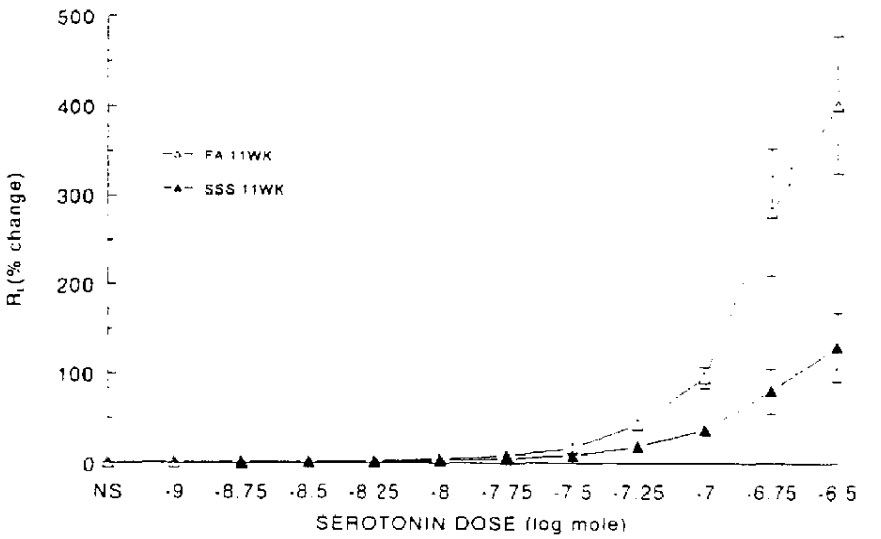

Fig. 2. Serotonin-induced changes of lung resistance (R_L,%) in rats exposed since day 2 of life to either filtered air (FA) (open triangles) or to sidestream smoke (SSS) (closed triangles) and studied at 11 weeks of life (n = 7 in each group). Serotonin increased R_L (P = 0.026), but less so in rats exposed to SSS (P = 0.004). Statistics by 1-way multivariate repeated measures ANOVA on the log transformed data.

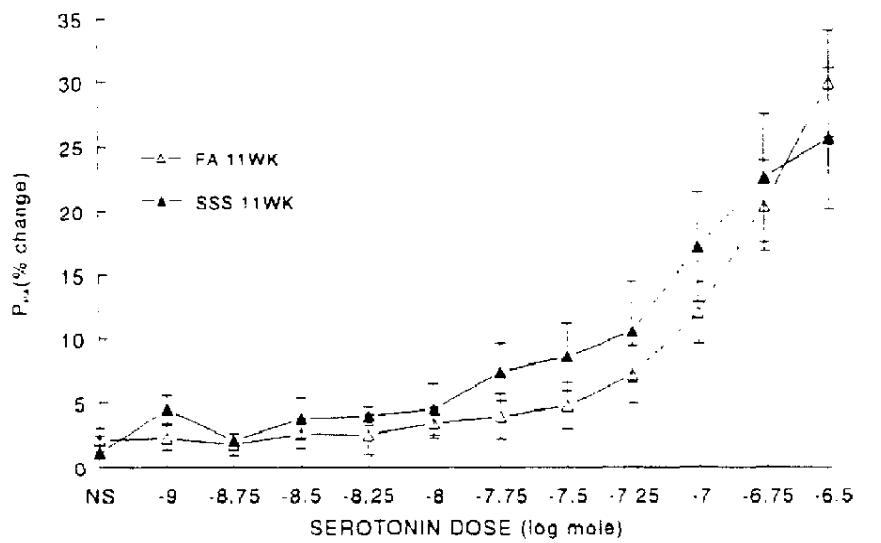

Fig. 3. Serotonin-induced changes in pulmonary artery pressure (P_{PA},%) in rats exposed since day 2 of life to either filtered air (FA) (open triangles) or to sidestream smoke (SSS) (closed triangles) and studied at 11 weeks of life (n = 7 in each group). Serotonin increased P_{PA} (P = 0.05), but SSS exposure did not alter this response (P = 0.63). Statistics by 1-way multivariate repeated measures ANOVA on the log transformed data.

The first explanation may be that the animals received an insufficient concentration of SSS to mimic human exposures. The concentration of SSS that was used in this study was in the high relevant range for human exposures (RSP = 1 mg/m^3 and CO = 6.45 ppm). A very smoky, poorly ventilated room has an RSP of 1 mg/m^3.[15] Although a more usual home environment concentration is 0.02 to 0.2 mg/m^3, RSP concentration increases logarith-

mically as the distance to a burning cigarette decreases and thus, a child tended to by a smoking caretaker may indeed be exposed to concentration of SSS used in this study.[15] Even this relatively high concentration of SSS may have been insufficient, however, if the rat is relatively resistant to SSS toxicity as it is to ozone toxicity.[16]

A second explanation may be that the SSS used in this study does not mimic ETS closely enough. ETS consists of both the smoldering smoke produced by the cigarette burning, and exhaled mainstream smoke[15]. Since we were unable to mimic the portion of ETS which represents exhaled mainstream smoke, the rats may have not received the appropriate concentration of an important component of ETS.

A third explanation may be that the mechanism by which the ETS causes lung problems in children may not be present in the Sprague Dawley rat. Such would be the case with C-fibers which do not appear to control airway tone in the Sprague–Dawley rat as they do in the guinea pig[17] (personal observations) yet are stimulated by mainstream tobacco smoke in rats as well as other species.[18–20]

A final possible explanation is that perhaps intrauterine exposure to smoke is more important than extrauterine exposure in inducing lung changes. Several studies have shown that the mother's smoking is more deleterious to their children than the father's smoking.[5,8,11,12] Since usually the mothers are the primary caretakers, this may represent simply a dose phenomenon. However, it is also possible that intrauterine exposure to smoke is more deleterious than postnatal exposure. Martinez et al.[10] showed that airway hyperreactivity was present in 70% of children whose mothers smoked during pregnancy as compared to only 29% of children whose mothers did not smoke during pregnancy. In that study, however, the effect of the mother's current smoking status could not be separated from the prenatal effects. Hanrahan et al.[21] showed that the expiratory flow at 50% of FRC in infants 4 weeks of age was 74.3 mL/s in infants born of continuous smokers vs 150 mL/s in infants born of nonsmokers. This was a highly statistically significant finding. Using multiple regression models, postnatal ETS exposure (exposure to smokers at least 2 hr twice a week) was not related to the pulmonary function of these infants. Although this study does not rule out a postnatal ETS effect on older children, or in infants with greater ETS exposure, it does suggest a major role for prenatal exposure to smoke in determining pulmonary function in children. Rats exposed to tobacco smoke in utero developed hypoplastic[22] and poorly developed[23] lungs. Whether intrauterine or postnatal exposure to smoke is more deleterious could be tested in this model by following postnatal pulmonary function and airway reactivity in rats exposed to smoke in utero, but not postnatally.

Regarding the acute SSS exposures, we did not know how acute SSS exposure would change muscarinic airway reactivity. Menon et al.[24] showed that 32% of asthmatic adults sensitive to ETS by history showed increased airway reactivity to methacholine when challenged with ETS acutely. On the other hand, Wiedemann et al.[25] showed that adult asthmatics exposed to ETS for 1 hr showed decreased airway reactivity to methacholine. We were unable to show any effect of acute ETS exposure on methacholine reactivity in the Sprague–Dawley rat.

Since we hypothesized that SSS exposure would increase airway reactivity to methacholine and/or serotonin, we were surprised to find that instead, SSS decreased airway reactivity to serotonin. The two most likely reasons for this decreased reactivity are (1) an increase in the serotonin uptake system or (2) down-regulation of the serotonin receptors. Serotonin is primarily taken up and degraded by pulmonary endothelial cells.[26,27] An increase in this uptake system would decrease the amount of circulating serotonin in contact with airway receptors. Indeed, another lung toxin, paraquat, has been shown to increase serotonin uptake.[27] However, if serotonin uptake were increased, both pulmonary artery and airway reactivity to serotonin should have been diminished. Since pulmonary artery reactivity was unchanged by SSS exposure, it is unlikely that the decreased airway reactivity to serotonin was due to an increase in serotinin uptake.

The other possibility is down-regulation of airway serotonin receptors due to chronic exposure to serotonin. Sparrow et al.[28] have shown that active smokers excrete more serotonin in their urine. As with most receptors, serotonin receptors become desensitized with chronic exposure.[29] The chronic increase in serotonin release could be from any of the serotonin-secreting cells: platelets,[30] mast cells,[31] and neuroendocrine cells[32]. Tobacco smoke has been shown to stimulate platelets[33] and to increase platelet activating factor concentration. However, platelets are probably not involved since it would again be expected that both pulmonary artery and airway serotonin receptors would be exposed to increased serotonin concentrations and demonstrate down-regulation. Tobacco smoke also activates C-fibers,[18] which in turn can activate mast cells.[35] This would preferentially affect the airways more than the pulmonary artery. Finally, SSS may increase the number or activity of airway neuroendocrine cells.

We found that methacholine reactivity was greater in the younger than in the older rats. Since baseline R_L did not differ at the two ages, a difference in underlying airway tone cannot explain the effect. The decrease in muscarinic airway reactivity with age is consistent with a corresponding decrease in muscarinic receptors in the rat.[36] Decreased airway reactivity with age may be species specific since airway reactivity to histamine and sub-

stance P increase with age in the guinea pig.[37] The increase in body weight and $C_{L,dyn}$ with growth were as expected. However, the decrease in lung weight/body weight ratio is at variance with the generally held belief that this value does not change with age.[38]

In conclusion, rats exposed postnatally to SSS did not appear to develop the effects reported in children raised in the homes of smokers: airway obstruction and increased nonspecific airway reactivity. This may have been due to insufficient SSS concentration in a resistant species, missing components in SSS which are present in ETS from exhaled mainstream smoke, or lack of in utero exposure of the pups as occurs in children of smoking mothers. The finding of SSS-induced decrease in serotonin reactivity raises interesting issues regarding the potential role of serotonin in ETS-induced lung disease.

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